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Observation of the Saxitoxin Producing Microalgae in the Kenyan Kilindini Port Creek Waters

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ABSTRACT

Surface water samples were collected from the Kilindini port creek in Kenya and analyzed for the presence, abundance and distribution of the saxitoxin producing microalgae species as from January, 2014 to July, 2014. Four species; *Alexandrium cohorticula*, *Alexandrium catenella*, *Gymnodium catenatum* and *Pyrodinium bahamense* out of the total 24 microalgae (*Alexandrium catenella*, *Ceratium furca*, *Dinophysis acuminata*, *Oscillatoria limosa*, *Ceratium lunula*, *Dictyocha fibula*, *Alexandrium cohorticula*, *Anabaena circinalis*, *Chaetocerus convolutes*, *Ostreopsis lenticularis*, *Thalassionema nitzchoides*, *Hemidiscus cuneiformis*, *Anabaena variabilis*, *Oscillatoria agardhii*, *Prorocentrum lima*, *Gonyaulax polygramma*, *Protoperidinium pallidum*, *Oscillatoria formosa*, *Skeletonema tropicum*, *Gymnodium catenatum*, *Ceratium lineatum*, *Ostreopsis ovata*, *Protoperidinium grande* and *Pyrodinium bahamense*) observed existing in the creek were saxitoxin producing. Four species were in abundance of 70% in the case of *Alexandrium catenella,* followed by *Gymnodium catenatum* at 22%, *Alexandrium cohorticula* at 7% and *Pyrodinium bahamense* being the least at only 1%. Their spatial distribution within the creek using a one-way ANOVA testing, revealed that *Alexandrium catenella* and *Gymnodium catenatum* significantly differed ($p<0.05$) in mean cell abundances±SEM at 95% level of significance within the sampled stations. *Alexandrium cohorticula* and *Pyrodinium bahamense* on the other hand did not differ.

Key words: Microalgae, species, abundance, distribution, saxitoxin

INTRODUCTION

Coastal port waters' biodiversity can be affected by the introduction of exotic microalgae species through the ships' ballast water (Globallast, 2000). Ships transport and discharge about 10^9 t of ballast water yearly throughout the globe facilitating the displacement of high organism's biomasses between ports (Globallast, 2000). This has caused significant ecological and economic impacts to the involved countries coastal waters (Mack *et al.*, 2000; Pimentel *et al.*, 2000; Carlton, 2001; Hewitt, 2003). The impacts are predicted to worsen with temperature disruptions and nutrient pollution increases brought about by the current climate change effects (Pearl and Huisman, 2008). The frequency and severity of marine algal bloom occurrences and seafood toxin poisonings emanating from the presence of such microalgae is therefore anticipated to greatly increase around the world in future.

The toxins are reported to have far reaching impacts ranging from increased human illnesses, huge economic losses as a result to mandatory closures of the fishing zones and increased fish mortalities (Costa *et al.*, 2009). Toxin monitoring and regulatory limit programmes have thus been established in areas prone to the occurrences of such microalgae and those experiencing recurrent fish kills (Van Egmond *et al.*, 1992; White, 1980; AFS., 1992; Svbobodova *et al.*, 1993). However, out of the great number of algal species existing worldwide, only a few have been associated with production of potent toxins currently divided into 5 major poison groups; Paralytic (PSP), Diarrhetic (DSP), Amnesic (ASP), Neurotoxin (NSP) shellfish poisonings and ciguatera fish poisoning. Consumption of these poisons from fish containing sufficient amounts has been linked to hepatoxicity, neurotoxicity and respiratory exposure effects (Ahmed, 1991; Falconer, 1999; Van Dolah, 2000).

The observed existing saxitoxin producing microalgae species in the Kenyan Kilindini port creek waters included; *Alexandrium catenella*, *Gymnodium catenatum*, *Alexandrium cohorticula* and *Pyrodinium bahamense*. The produced saxitoxin contain tetrahydropurine compounds composed of 2 guanidine moieties fused together in a stable azaketal linkage (Fig. 1) from which, it derives its toxic potency. When ingested in appreciable amounts from shellfish, it causes Paralytic (PSP) shellfish poisoning (Lambert *et al.*, 1994). Therefore, the observation of these saxitoxin producing microalgae species in the creek waters serve as an early warning of possible future bloom occurrences with subsequent potential impacts. Furthermore, the country has also received EU bans associated with fish safety with the most recent one being in the year 2000. The purpose of this study was therefore to determine the presence, abundance and spatial distribution of the saxitoxin (PSP) producing microalgae species in the Kenyan Kilindini port creek areas in order to help in developing monitoring and mapping of such toxic microalgae in the Kenyan coastal waters.

MATERIALS AND METHODS

Study site: The samples were obtained from the Kenyan-Kilindini port creek area (04° 03'11" South and 039°37'31" East), which is one of the two main tidal mangrove-fringed creeks found in Mombasa District, Kenya suitable for bivalve culture (Fig. 2). Water depths in the creek vary from about 30 m in the Port area along Kilindini channel to less than 14 m at Kipevu basin. Depths are however, less than 6 m in the tidal channels fringing the mangroves. The creek receives freshwater and terrigenous sediments from three seasonal rivers namely, Mwachi, Chasimba and Mambona (Kitheka, 1996), with the main environmental problems being ballast water discharge, siltation, oil pollution, eutrophication and degradation of the mangrove ecosystems.

Sample collection procedures: Sampling of water was carried out in stations 1-3 (Fig. 2). Each station was visited three times during the seven months of study. Surface water samples were collected in triplicates from the sampling stations within the creek for both qualitative and quantitative analysis. A boat was used to access the sites. The surface water samples were collected
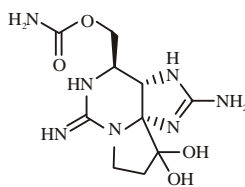

Fig. 1: Chemical structure of saxitoxin (A marine paralytic shellfish poisoning algal toxin that impact human health)
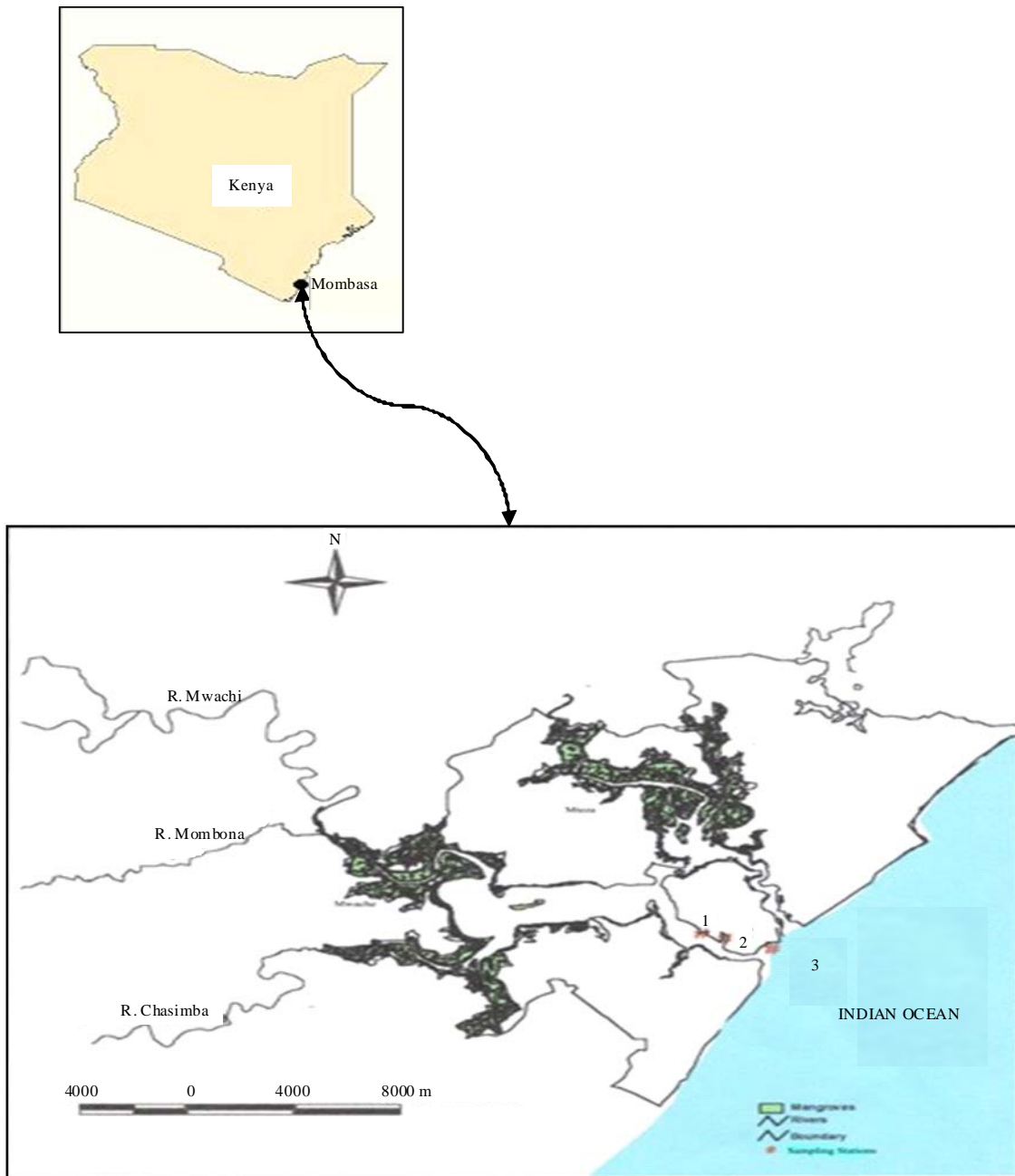
Fig. 2: A map of Mombasa port creek showing the influencing rivers, associated mangrove forests and sampling stations denoted by numbers 1-3

using a 20 L bucket of which its water content was passed through 20 µm mesh-size plankton net for concentration to 100 mL. The resultant concentrated plankton was then transferred to sample bottles labeled with date, sampling station and immediately preserved in Lugol's solution and transported to the laboratory for identification and enumeration. The qualitative samples were immediately stored after collection in a cooler box without preservation in Lugol's and transported to the Laboratory for gross examination.

Water quality parameter samples: Surface water quality parameters (pH, temperature, salinity and turbidity) were measured *in situ* at each station visited. The pH was measured using an electronic pH probe (pH Testr 2; Aquatic Eco-Systems, Inc., USA), temperature using an YSI Model 550 A and salinity using a hand-held refractometer (all from Yellow Springs Instruments, Yellow Springs, OH, USA). Turbidity was measured using a locally made Secchii disc. Nutrient water samples for dissolved inorganic Nitrates (NO_3), Phosphates (PO_4) and Ammonia (NH_3) were aseptically collected from each station and stored in a pre-cooled ice-box and transported to the laboratory for analysis by using Parsons *et al.* (1984) methods.

Sample analysis procedures: In the Laboratory, 1 mL aliquots of samples preserved in Lugol's iodine solution was pipetted into Sedgewick-Rafter counting cells and observed under an inverted microscope and the counts of all seen phytoplankton species recorded. An Intergovernmental Oceanographic Commission (IOC) of UNESCO "Identifying Marine Phytoplankton" manual and guides no. 41 on "Potentially Harmful Microalgae of the Western Indian Ocean", (Hansen *et al.*, 2001) and a "Phytoplankton Identification Catalogue of Saldanha Bay, South Africa", (Botes, 2003) were used for species identifications.

Determination of relative abundance of the saxitoxin producing microalgae: The quantity of saxitoxin producing microalgae in the samples was evaluated using an inverted microscope and sedimentation chamber method adopted from Utermohl (1958). This is a useful procedure for counting algae in low concentrations of less than 10^2-10^4 cells L^{-1}. The 95% confidence limits of cells L^{-1} of the saxitoxin producing microalgae species in the stations was then calculated using the Poisson distribution method: $\pm(2\times\sqrt{n}\times100\%)/n = \pm200\%/\sqrt{n}$, in which n is the counted Saxitoxin producing microalgae.

Statistical analyses: Data were first tested for normality and homogeneity of variances in order to apply parametric statistics and where the criterion was not met, data was arcsine transformed and "MINITAB version 14" software used to perform the analyses. Mean phytoplankton species abundances in the stations within the creek was tested using a one-way ANOVA at the significance level of $\alpha = 0.05$. The mean saxitoxin producing microalgae species abundances in the stations was also compared using ANOVA. The existing correlation between the saxitoxin producing micro algal species abundance and the nutrient's level within the creek was also tested using Spearman's rank correlation test. The physico-chemical parameter relationship with the observed saxitoxin producing species abundances was also tested using the General Linear Model (GLM) multiway ANOVA.

RESULTS

Composition and abundance of all the phytoplankton species observed in the creek: In general, a total of 24 phytoplankton species; *Alexandrium catenella, Ceratium furca, Dinophysis acuminata, Oscillatoria limosa, Ceratium lunula, Dictyocha fibula, Alexandrium cohorticula, Anabaena circinalis, Chaetocerus convolutes, Ostreopsis lenticularis, Thalassionema nitzchoides, Hemidiscus cuneiformis, Anabaena variabilis, Oscillatoria agardhii, Prorocentrum lima, Gonyaulax polygramma, Protoperidinium pallidum, Oscillatoria formosa, Skeletonema tropicum, Gymnodium catenatum, Ceratium lineatum, Ostreopsis ovata, Protoperidinium grande* and *Pyrodinium bahamense* were observed existing within the creek (Table 1). Their mean abundances

Table 1: Mean abundance (numbers±SE) of all the phytoplankton species analyzed within the creek

Species	Mean abundances			ANOVA	
	Station 1	Station 2	Station 3	F	p
Alexandrium catenella	0	0	107±13	57.394	0.000
Alexandrium cohorticula	10±4	0	0	1.000	0.422
Anabaena circinalis	10±4	0	0	3.162	0.115
Anabaena variabilis	3±2	0	0	1.000	0.422
Ceratium furca	50±9	40±8	27±6	0.104	0.903
Ceratium lineatum	0	0	20±5	1.000	0.422
Ceratium lunula	23±6	0	0	1.000	0.422
Chaetocerus convolutus	10±4	0	0	1.000	0.422
Dictyocha fibula	20±5	33±7	13±4	1.692	0.261
Dinophysis acuminata	0	33±7	50±9	29.464	0.001
Gonyaulax polygramma	0	13±4	13±4	10.343	0.011
Gymnodium catenatum	0	0	33±7	168.253	0.000
Hemidiscus cuneiformis	7±3	0	0	1.000	0.422
Oscillatoria agardhii	0	30±7	0	1.000	0.422
Oscillatoria formosa	0	10±4	0	0.500	0.630
Oscillatoria limosa	40±8	40±8	43±8	0.100	0.906
Ostreopsis lenticularis	10±4	0	0	1.000	0.422
Ostreopsis ovata	0	0	20±5	1.000	0.422
Prorocentrum lima	0	23±6	20±5	3.584	0.095
Protoperidinium grande	0	0	7±3	1.000	0.422
Protoperidinium pallidum	0	13±4	0	1.000	0.422
Pyrodinium bahamense	0	0	3±2	1.000	0.422
Skeletonema tropicum	0	10±4	0	1.000	0.422
Thalassionema nitzchoides	10±4	0	0	1.000	0.422

ranged from 3±2-107±13 cells L^{-1} and the most common species in all the three creek stations were *Ceratium furca*, *Oscillatoria limosa* and *Dictyocha fibula*.

The most abundant species (cells L^{-1}±SE) in station 1 were; *Ceratium furca* (50±9), *Oscillatoria limosa* (40±8), *Ceratium lunula* (23±6), *Dictyocha fibula* (20±5), *Alexandrium cohorticula*, *Anabaena circinalis*, *Chaetocerus convolutes*, *Ostreopsis lenticularis*, *Thalassionema nitzchoides* (all with10±4), *Hemidiscus cuneiformis* (7±3) and *Anabaena variabilis* (3±2). Station 2 had the following species; *Ceratium furca*, *Oscillatoria limosa* (both having 40±8), *Dictyocha fibula*, *Dinophysis acuminata* (33±7), *Oscillatoria agardhii* (30±7), *Prorocentrum lima* (23±6), *Gonyaulax polygramma*, *Protoperidinium pallidum* (all with 13±4), *Oscillatoria formosa* and *Skeletonema tropicum* both with densities of 10±4 cells L^{-1}. In station 3; *Alexandrium catenella* (107±13), *Dinophysis acuminata* (50±9), *Oscillatoria limosa* (43±8), *Gymnodium catenatum* (33±7), *Ceratium furca* (27±6), *Ceratium lineatum*, *Ostreopsis ovata*, *Prorocentrum lima* (all having 20±5), *Dictyocha fibula*, *Gonyaulax polygramma* (both with 13±4), *Protoperdinium grande* (7±3) and *Pyrodinium bahamense* (3±2) cells L^{-1}, respectively (Table 1).

Four of the species comprising *Alexandrium catenella, Dinophysis acuminata, Gonyaulax polygramma* and *Gymnodium catenatum*), were found having significant differences in their abundance and distributions within the stations (p<0.05) (Table 1).

Mean abundances of the observed saxitoxin producing micro algal species analyzed in the creek: Four saxitoxin producing microalgae species; *Alexandrium cohorticula, Alexandrium catenella, Gymnodium catenatum* and *Pyrodinium bahamense* were identified within the creek (Table 1). None of the species was observed in station 2, whereas, only *Alexandrium cohorticula* was observed in station 1. Their overall mean abundances in the creek stations ranged from 10±4 cells L^{-1} in case of *Alexandrium cohorticula* in station 1. Station 3 had 3 of the saxitoxin
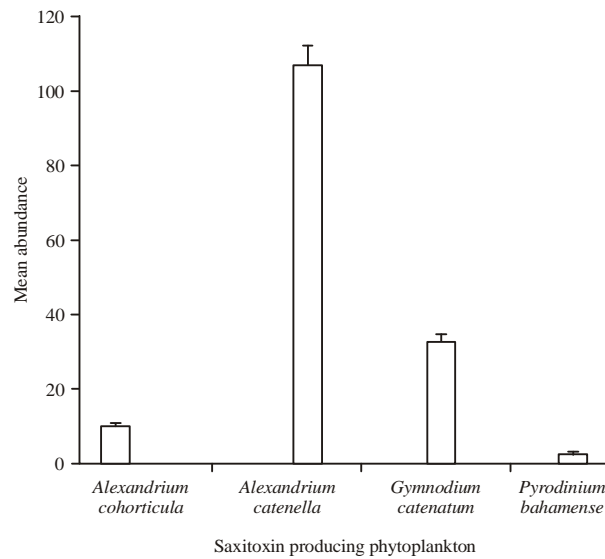
Fig. 3: Overall mean abundance variations (cell numbers±SE) of the saxitoxin producing microalgae species within the creek

species namely; *Alexandrium catenella, Gymnodium catenatum* and *Pyrodinium bahamense* which their abundances ranged from 3±2 in case of *Pyrodinium bahamense;* 33±7 for *Gymnodium catenatum;* and 107±13 cells L^{-1} in case of *Alexandrium catenella* (Table 1).

The mean overall abundances (Cell Numbers±SE) of the observed Saxitoxin producing microalgae species within the creek was found least for the *Pyrodinium bahamense,* followed by *Alexandrium cohorticula,* then *Gymnodium catenatum and Alexandrium catenella* being the most abundant (Fig. 3).

Two of the species; *Alexandrium catenella* and *Gymnodium catenatum*, were found having significant variations of their overall mean abundances within the creek ($p < 0.05$) following the ANOVA testing (Table 1). The other two (*Alexandrium cohorticula* and *Pyrodinium bahamense*) had no significant variations (Table 1).

The existing relationship between the observed Saxitoxin producing microalgae species abundances with nutrient levels within the creek: The non-parametric Spearman's rank correlation test showed that the species, *Alexandrium catenella, Gymnodium catenatum* and *Pyrodinium bahamense* had a non-significant positive relationship with phosphate levels in the creek (Table 2). *Alexandrium cohorticula* on the other had a non-significant negative relationship with phosphates in the creek.

The relationship among *Alexandrium catenella, Gymnodium catenatum* and *Pyrodinium bahamense* and nitrate levels in the creek was negative and not significant (Table 2). On the other hand, a positive but non-significant relationship existed between *Alexandrium cohorticula* and nitrates.

However, a positive significant relationship existed between *Alexandrium catenella* (rho = 0.78) and *Gymnodium catenatum* (rho = 0.78) with Ammonia concentration (Table 2). *Pyrodinium bahamense* had a positive non-significant relationship whereas *Alexandrium cohorticula* had a non-significant negative relationship with Ammonia levels in the creek (Table 2).

Table 2: Existing relationship between the observed saxitoxin producing microalgae species abundances with the nutrient levels within the creek

Saxitoxin producing species	Nutrient parameters					
	Phosphates		Nitrates		Ammonia	
	Correlation index (rho)	p-value	Correlation index (rho)	p-value	Correlation index (rho)	p-value
Alexandrium cohorticula	-0.413	0.270	0.411	0.272	-0.411	0.272
Alexandrium catenella	0.547	0.128	-0.119	0.761	0.782	0.013
Gymnodium catenatum	0.520	0.151	-0.189	0.626	0.777	0.014
Pyrodinium bahamense	0.550	0.125	-0.137	0.725	0.274	0.476

Table 3: Observed mean values of the physico-chemical parameters

Parameters	Observed mean values per stations				ANOVA	
	Station 1	Station 2	Station 3	Min-Max	F	p
NO_3 (mg L^{-1})	0.26±0.05	0.55±0.05	0.81±0.05	0.26-0.81	12.332	0.007
PO_4 (mg L^{-1})	0.02±0.05	0.02±0.05	0.02±0.05	0.02-0.02	1.115	0.387
NH_3 (mg L^{-1})	0.08±0.05	0.08±0.05	0.10±0.05	0.08-0.10	6.725	0.029
Salinity (%)	36.20±0.5	38.10±0.5	39.30±0.5	36.20-39.3	2.841	0.136
Temperature (°C)	28.60±0.5	29.90±0.5	30.60±0.5	28.60-30.6	18.540	0.003
pH	7.90±0.05	8.10±0.05	8.50±0.05	7.90-8.5	12.043	0.008
Secchii disc (m)	0.50±0.1	0.90±0.1	2.00±0.1	0.50-2.0	19.642	0.002

Creek's water quality parameter levels: The mean water quality parameters such as NO_3, PO_4, NH_3, salinity (%), pH, temperature (°C) and water transparency (i.e., secchii disc depth) observed during the period of study in the sampling stations are shown in Table 3.

Nitrate concentrations (mg L^{-1}) ranged from 0.26±0.05 (Station 1), 0.55±0.05 (Station 2), to 0.81±0.05 (Station 3), while that of Phosphates (mg L^{-1}) ranged at 0.02±0.05 in all the stations. Ammonia concentrations (mg L^{-1}) had highest values in station 3 (0.10±0.05) and lowest values in both stations 1 and 2 (0.08±0.05). Salinity (%) values were nearly equal between the stations ranging from 36.2±0.5-39.3±0.5. Temperature (°C) had lower values of 28.6±0.5, 29.9±0.5 in stations 1 and 2, respectively and a higher value of 30.6±0.5 in station 3. The pH had values of 7.9±0.05 in station 1, 8.1±0.05 in station 2 and 8.5±0.05 in station 3. Secchii disc measurements (m) were however 0.5±0.1 in station 1, 0.9±0.1 in station 2 and 2.0±0.1 in station 3 (Table 3).

ANOVA tests indicated significant differences between stations in the values of Nitrates (NO_3), Ammonia (NH_3), Temperature (°C), pH and Secchii depth measurements ($p < 0.05$) (Table 3).

DISCUSSION

The microscopic analysis of the sample obtained during the study, revealed the presence of 4 saxitoxin producing microalgae species; *Alexandrium catenella*, *Alexandrium cohorticula*, *Gymnodinium catenatum* and *Pyrodinium bahamense*. Their abundance numbers ranged from 3±2-107±13 cells L^{-1}. However, none of the saxitoxin producing phytoplankton species was observed in station 2 of the creek. The cause of disparity could not be immediately identified as all had no significant difference in terms of water quality (Table 3). Further studies therefore need to be undertaken to determine if it could have been probably due to the purifying effects of the mangrove forests covering the upper areas of the creek (Fig. 2) or due to the dilution effects of the received freshwater and terrigenous sediments from the three seasonal rivers into the creek. The high numbers observed in station 3 or the 2 species (*Alexandrium catenella* and *Gymnodium catenatum*), could be due to the globallast water discharge activities in the port (Seliger, 1993).

Further studies are however required to determine the real factors affecting the distribution of these toxic producing algae within the creek. However, the study has shown that saxitoxin producing microalgae exist within the creek.

The analysis also revealed the presence of 24 microalgae species (Table 1). This high algal abundance can directly influence water quality and may more importantly contribute to heavy metals pollutant transfer through the food chain, posing serious threats to animals and humans through biomagnifications (Munga *et al.*, 1992). Water pollution levels can also be accurately identified by analyzing the species abundance, physiological, biological responses and residue contents (Cosper *et al.*, 1989). However, they may not only be significant for biomonitoring studies but could also be a useful phytoremediation technology to restore water quality due to their high bioaccumulation abilities (Munga *et al.*, 1992).

Looking at the occurrence of the saxitoxin producing microalgae in relation to other toxin producing species like the Domoic acid producing microalgae within the creek; indicated that in station 1 only one saxitoxin producing species (Alexandrium cohorticula) was recorded (Table 1), none of the Domoic acid producing microalgae species such as *Dinophysis acuminata* and *Gonyaulax polygramma* was recorded in this station which was most adjacent to the mangrove vegetation cover and is also influenced by the three river discharges (Fig. 2). However, in station 2, where no saxitoxin producing microalgae was recorded (Fig. 2); two of the Domoic acid producing microalgae species; *Dinophysis acuminata* and *Gonyaulax polygramma* were recorded (Table 1). This phenomenon needs to be investigated further to determine, whether the mangrove or the rivers had a role in this happening. The investigation can also be modeled to include the usage of toxin concentration equipment determinants, such as micro-beta, used in the receptor binding assay method for assuring that the toxins had not been produced at the sites at all.

The non-parametric spearman's rank correlation tests to determine the existing relationships between the abundances of the saxitoxin producing microalgae species with the observed nutrient levels within the creek revealed that *Alexandrium catenella, Gymnodium catenatum* and *Pyrodinium bahamense* had a non-significant positive relationship between them and phosphate values. This indicates that they have no strong relationship with phosphates presence. *Alexandrium cohorticula* on the other hand, had a non-significant negative relationship with phosphates indicating that they were not dependent on the phosphates presence (Table 2). This non-significant relationship between them and nitrate values was also recorded for *Alexandrium catenella, Gymnodium catenatum, Pyrodinium bahamense* (all with negative relationships) and a positive non-significant one for *Alexandrium cohorticula* (Table 2). However, this changed in respect to ammonia, where there was a positive significant relationship for *Alexandrium catenella* and *Gymnodium catenatum* indicating their strong relationship with ammonia within the creek (Table 2). *Pyrodinium bahamense* also had a positive non-significant relationship with ammonia. *Alexandrium cohorticula* had a non-significant negative relationship. The mean water quality parameters tested using the General Linear Model (GLM) multiway ANOVA to examine their influences within the creek; revealed that only Ammonia had a positive significant influences on the abundance and distribution of *Alexandrium catenella* (rho = 0.78) and *Gymnodium catenatum* (rho = 0.78) (Table 2).

The observation shows that there is a potential risk to human health as well as economic loss to the Kenyan fishery and aquaculture industry that may result from algal toxins. Therefore, there is need to set up regular rapid monitoring programmes similar to that suggested by Powell and

Doucette (1999) for their detection and formulation of impact reduction measures along the Kenyan Exclusive Economic Zones (EEZ) coastline. In addition, effective education, dissemination and communication of the available information to the users and stakeholders are necessary to ease regulation, harvesting and use of the Kenyan fishery resources.

CONCLUSION

The observation has revealed the presence, abundance and distribution of some potential toxin producing microalgae species that have been documented elsewhere in the world as causative agents for saxitoxins within the Kenyan port creek waters. This poses greater probability of toxic algal event outbreaks occurring in future. Therefore the resultant potential economic loss to the Kenyan fishery and human health impacts cannot be underestimated.

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